

Attorney Docket No.: T4903.CIP (UT-0003)
Inventors: Rao et al.
Serial No.: 09/073,881
Filing Date: May 6, 1998
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This listing of the claims will replace all prior versions and listings of claims in the application:

Listing of the claims:

Claim 1: (currently amended) A method for generating mammalian neural crest stem cells comprising:

(a) obtaining mammalian, p75 immunonegative neuroepithelial stem cells derived from the neural tube from a mammalian embryo at a stage of embryonic development after closure of the neural tube by:

(i) removing a sample of neural tube tissue from a mammal at a stage of embryonic development after closure of the neural tube;

(ii) dissociating cells comprising the sample of neural tube tissue removed from the mammal; and

(iii) plating the dissociated cells in feeder-cell-independent culture on a substratum and in a media comprising fibroblast growth factor and chick embryo extract; and

(iv) identifying p75 immunonegative cells via immunohistochemistry and obtaining the mammalian, p75 immunonegative neuroepithelial stem cells;

(b) harvesting the mammalian, p75 immunonegative

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neuroepithelial stem cells by trypsinization; and

(c) replating the harvested mammalian, p75 immunonegative neuroepithelial stem cells onto a fibronectin substrate and in a media comprising chick embryo extract, NGF, EGF and EGF to generate p75 immunoreactive neural crest stem cells.

Claims 2-14 (canceled)

Claim 15 (currently amended): A method for generating rat neural crest stem cells comprising:

(a) obtaining rat, p75 immunonegative neuroepithelial stem cells derived from the neural tube from a rat embryo at a stage of embryonic development after closure of the neural tube by:

(i) removing a sample of neural tube tissue from a rat at a stage of embryonic development after closure of the neural tube;

(ii) dissociating cells comprising the sample of neural tube tissue removed from the rat; and

(iii) plating the dissociated cells in feeder-cell-independent culture on a substratum and in a media comprising fibroblast growth factor and chick embryo extract so that rat neuroepithelial stem cells are obtained; and

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(iv) identifying p75 immunonegative cells via immunohistochemistry and obtaining the mammalian, p75 immunonegative neuroepithelial stem cells;

(b) harvesting the rat, p75 immunonegative neuroepithelial stem cells by trypsinization; and

(c) replating the harvested rat, p75 immunonegative neuroepithelial stem cells onto a fibronectin substrate and in a media comprising chick embryo extract, NGF, FGF and EGF to generate rat, p75 immunoreactive, neural crest stem cells.

Claim 16 (canceled)

Claim 17 (previously added): A method for isolating mammalian neural crest stem cells comprising:

(a) generating neural crest stem cells in accordance with the method of claim 1; and

(b) isolating the neural crest stem cells via antibody capture with an antibody against neurotrophin receptor p75.

Claim 18 (previously added): A method for isolating rat neural crest stem cells comprising:

(a) generating rat neural crest stem cells in accordance with

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the method of claim 15; and

- (b) isolating the rat neural crest stem cells via antibody capture with an antibody against neurotrophin receptor p75.